**AS Unit 1: Basic Biochemistry and Cell Organisation**

| Name: | Date: |
|---|---|

## Topic 1.4 Biological Reactions are Catalysed by Enzymes – Page 1

From the syllabus:

(a) metabolism as a series of enzyme controlled reactions

(b) the protein nature of enzymes

(c) enzymes acting intracellularly or extracellularly

(d) active sites, interpreted in terms of three dimensional structure

(e) the theory of induced fit as illustrated by lysozyme

(f) the meaning of catalysis; the lowering of the activation energy

(g) the influence of temperature, pH, substrate and enzyme concentration on rate of activity and inactivation and denaturation of enzymes and the importance of buffers for maintaining a constant pH

(h) the principles of competitive and non-competitive inhibition

(i) the importance of immobilised enzymes and that industrial processes use immobilised enzymes, allowing enzyme reuse and improving stability

**SPECIFIED PRACTICAL WORK**

- Investigation into the effect of temperature or pH on enzyme activity
- Investigation into the effect of enzyme or substrate concentration on enzyme activity

### I. Introduction to Enzymes

| | | Completed |
|---|---|---|
| 1. | Go through the PowerPoint on Enzymes | |
| 2. | Read the following:<br>• Rowlands p45-50<br>• Toole p35-44<br>• Hand out Enzyme Activity<br>• Hand out Factors Affecting Enzymes<br>• Hand out Enzyme Catalysis | |
| 3. | Complete the tasks on pages | |

End of topic check list for BIOLOGICAL REACTIONS ARE CATALYSED BY ENZYMES

Tick as appropriate:
RED: I do not know about this
AMBER: I have heard about this but have not learned this yet. I am unsure on this.
GREEN: I have heard about this and I have learned this. I am confident about this.

| | Topic | RED | AMBER | GREEN |
|---|---|---|---|---|
| 1. | The general characteristics of enzymes are due to their biochemical nature as globular proteins, showing specificity, requiring certain conditions and with a mode of action lowering the activation energy of a reaction. | | | |
| 2. | The substrate binds to part of the protein called the active site. | | | |
| 3. | Understand the action of enzymes explained in relation to enzyme structure - lock and key hypothesis; the theory of induced fit, whereby the specific substrate for the enzyme alters the shape of the active site on binding as illustrated by lysozyme | | | |
| 4. | Enzymes are proteins made inside living cells but may act inside the cell (intracellular) or outside (extracellular) such as the digestive enzymes of the alimentary canal. | | | |
| 5. | The rate of an enzyme catalysed reaction increases with increasing temperature due to increased frequency of collisions as shown by a graph. | | | |
| 6. | The rate of an enzyme catalysed reaction will vary with changes in pH as shown by a graph. | | | |
| 7. | The rate of an enzyme catalysed reaction will vary with changes in enzyme concentration as shown by a graph. | | | |
| 8. | The rate of an enzyme catalysed reaction will vary with changes in substrate concentration as shown by a graph. | | | |
| 9. | Know about the need for buffers in enzyme experiments and the requirement for adequate controls. | | | |
| 10. | Environmental conditions such as temperature and pH change the three dimensional structure of enzyme molecules. Bonds are broken and hence the configuration of the active site is altered. | | | |
| 11. | High temperatures and extreme changes in pH cause permanent change in protein structure, causing denaturation. | | | |
| 12. | Small changes in pH cause small reversible changes in enzyme structure, extreme changes causing inactivation. | | | |
| 13. | Inhibition is when enzyme action is slowed down or stopped by another substance. | | | |
| 14. | Enzyme inhibition may be competitive whereby an inhibitor, which is structurally similar to the substrate, associates with the enzyme active site. If the substrate concentration is increased so will the rate of reaction. | | | |
| 15. | Non competitive inhibition involves an inhibitor combining away from the active site often altering the enzyme shape as illustrated by potassium cyanide. The | | | |

| | | | |
|---|---|---|---|
| rate of reaction is unaffected by substrate concentration. | | | |
| 16. When a mixture is passed over the enzyme a reaction occurs. The energy released is proportional to the concentration of the substrate and is converted into electrical impulses. Consequently an accurate digital display of concentration is produced e.g. glucose oxidase electrode detects glucose in blood. | | | |
| 17. An enzyme can detect the presence of its substrate even in very low concentrations. | | | |
| 18. The enzyme is immobilised so its structure is stabilised in an inert support e.g. on alginate beads or gel membrane. | | | |
| 19. Industrially an immobilised enzyme can be recovered for re-use. Therefore, a small amount of enzyme may be used to carry out a large-scale reaction. | | | |

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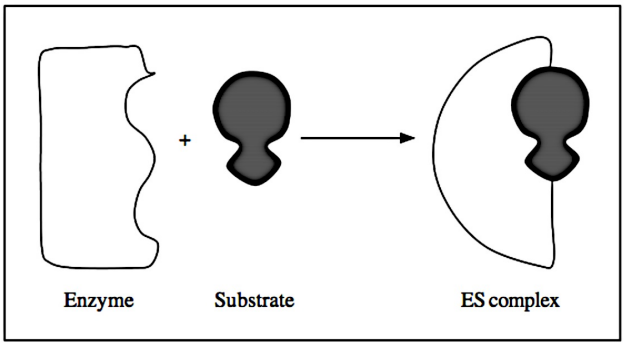
# Factors Affecting Enzyme Activity

Enzymes are globular proteins which act as biological catalysts. This means that they speed up the rate of reaction by lowering the activation energy, that is the energy required to break bonds. Enzymes are a complex tertiary and sometimes quaternary shape and catalyse reactions by forming a complex (known as the enzyme substrate complex) at a specific region of the enzyme called the active site.

Enzyme + substrate → enzyme substrate complex → product

Enzymes are specific; any individual enzyme can usually only catalyse one particular reaction. The **induced fit hypothesis** has been put forward to explain how enzymes work. The key points of the induced fit hypothesis are as follows (Fig1):

**Fig 1. Induced fit hypothesis**




1. Substrate approaches the active site of the enzyme.
2. The shape of the active site then changes to fit precisely around the substrate – in other words, the substrate **induces** the active site to change shape.
3. The reaction is catalysed and products form.
4. The products are a different shape from the substrate and therefore diffuse away from the active site. As they do, the active site reverts to its original shape.

**Factors affecting enzyme activity**

**1. Temperature**

Enzymes have an optimum temperature – this is the temperature at which they work most rapidly. Below the optimum temperature, increasing temperature will increase the rate of the reaction. This is because temperature increases the kinetic energy of the system, effectively increasing the number of collisions between the substrate and the enzyme's active site.

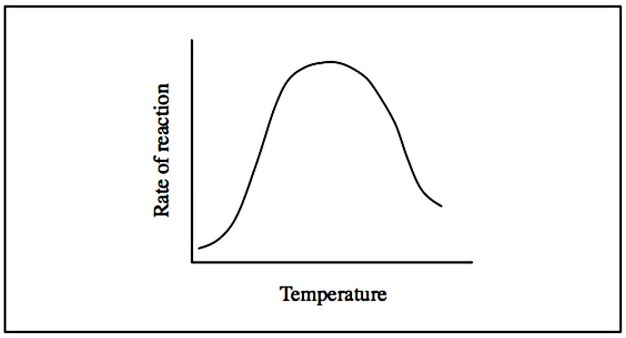
Temperatures above the optimum will lead to **denaturation**. This occurs because the hydrogen bonds and disulphide bridges which maintain the shape of the active site are broken. Thus, enzyme substrate complexes can no longer be formed.

The effect of temperature on the rate of a chemical reaction is described by the term "temperature coefficient" ($Q_{10}$).

$$Q_{10} = \frac{\text{rate of reaction at T + 10°C}}{\text{rate of reaction at T°C}}$$

Many enzymes have a $Q_{10}$ of between 2 and 3. In other words, provided that the temperature is not so high that it causes denaturation, an increase in temperature of 10°C will speed up the reaction by a factor of 2-3, that is it will double or treble it (Fig 2).

**Fig 2. Effect of temperature on enzyme activity**

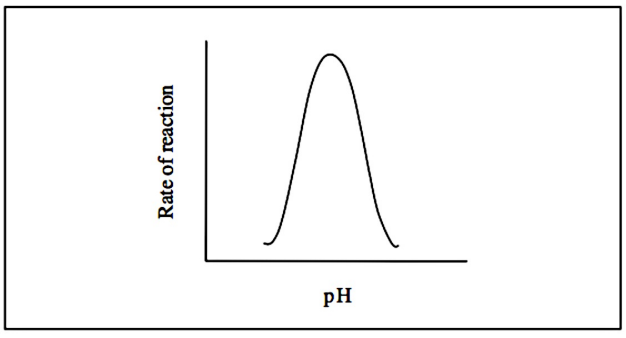



**2. pH**

The effect of a change in pH on enzyme activity is shown in Fig 3. As with temperature, each enzyme has an optimum pH. If pH increases or decreases much beyond this optimum, the ionisation of groups at the active site and on the substrate may change, effectively slowing or preventing the formation of the enzyme substrate complex. At extreme pH, the bonds which maintain the tertiary structure – hence the active site – are disrupted and the enzyme is irreversibly denatured.

Since most human enzymes are intracellular, most have a pH optimum of 7.3-7.4. However, pepsin, which works in the acidic environment of the stomach, has an optimum of 2.4 (Fig 3).

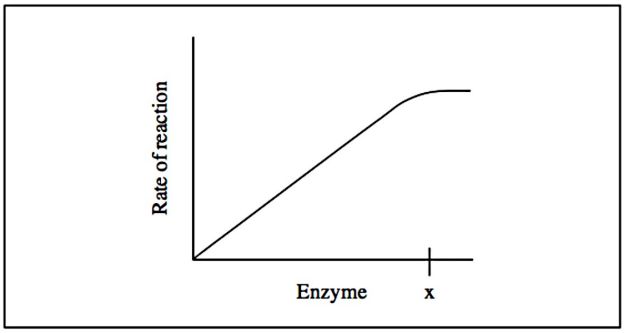
**Fig 3. Effect of pH on enzyme activity**



### 3. Enzyme concentration

The effect of enzyme concentration on the rate of reaction is shown in Fig 4. At low enzyme concentrations there are more substrate molecules than there are available active sites. Increasing the number of active sites by increasing the concentration of the enzyme, therefore, effectively increases the rate of the reaction. Eventually, at point x, increasing the enzyme concentration has no effect on the rate of reaction. This is because it is now the number of substrate molecules which has become the limiting factor.

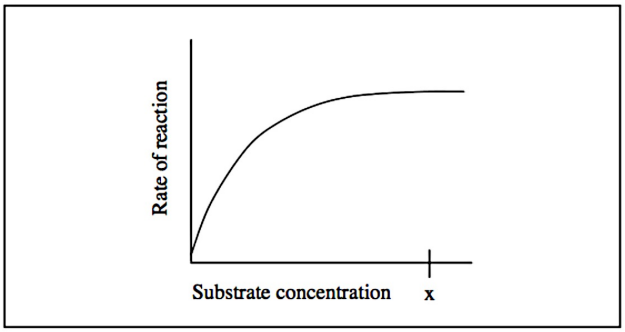
**Fig 4. Effect of enzyme concentration on enzyme activity**




### 4. Substrate concentration

Fig 5 shows the effect of substrate concentration on the rate of reaction.

**Fig 5. Effect of substrate concentration on enzyme activity**




At low substrate concentration the reaction proceeds slowly. This is because there are not enough substrate molecules to occupy all of the active sites on the enzyme. As substrate concentration increases, the rate increases because there are more enzyme substrate complexes formed. At point x, however, increasing the substrate concentration will have no further effect on the rate of reaction. This is because all of the enzyme's active sites are now occupied by substrate molecules – increasing the substrate concentration further will have no effect, because no more enzyme substrate complexes can form. The rate of reaction now depends on the turnover rate of the enzyme, i.e. the number of substrate molecules transformed by one molecule of enzyme per second. Carbonic anhydrase has the highest turnover rate of any known enzyme (Table 1).

**Table 1. Enzyme turnover rates**

| Enzyme | Turnover rate |
|---|---|
| Carbonic anhydrase | $36 \times 10^6$ |
| Catalase | $5.6 \times 10^6$ |
| Lysozyme | 60 |

### 5. Cofactors

Many enzymes require cofactors to function properly. There are three main types of cofactor; co-enzymes, inorganic ions and prosthetic groups.

1. **Coenzymes** are organic molecules which often contain a vitamin molecule as part of their structure. Coenzymes become loosely bound to the enzyme and move away from the enzyme once the reaction is completed. One coenzyme, e.g. $NAD^+$ may react with many different enzymes in many different types of reaction. $NAD^+$ transfers hydrogen in reactions involving dehydrogenase enzymes.
2. **Inorganic** metal ions are also known as enzyme activators. They change the charge in the active site, enabling the enzyme substrate complex to form. Some become intimately bound to the enzyme, e.g. $Fe^{2+}$ in catalase. Most others accelerate the binding between the enzyme and the substrate, e.g. $Mg^{2+}$ in phosphotransferases.
3. **Prosthetic** groups are coenzymes that bind permanently to the enzyme molecule and remain there even after the reactions are complete, e.g. FAD (flavin adenine dinucleotide). Like $NAD^+$ it carries hydrogen atoms, this time with oxidase enzymes.

### 6. Inhibitors

Inhibitors slow down the rate of reaction. As such, they are an essential form of cellular control, allowing enzyme reaction rate to be slowed when necessary. Some enzymes are inhibited by the end product of the reaction they catalyse (see Factsheet 31 Enzyme control of metabolic pathways).

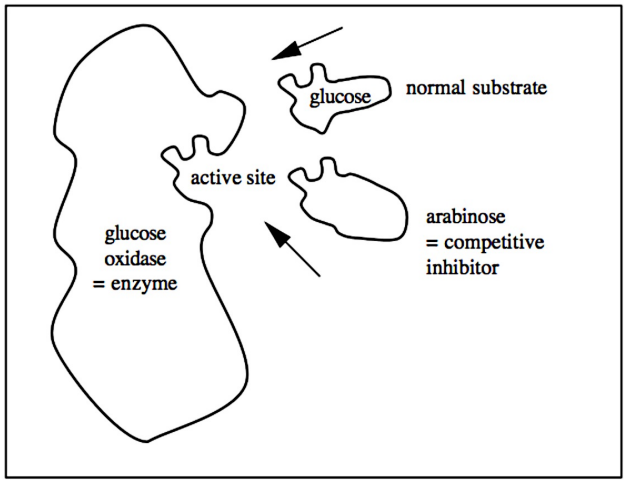
#### (a) Reversible inhibitors

There are two types of reversible inhibitor:

- competitive reversible inhibitor
- non-competitive reversible inhibitor

**Competitive reversible inhibitors** are structurally similar to the normal substrate and compete with the normal substrate for the active sites (see Fig 6).

**Fig 6. Competitive reversible inhibition**

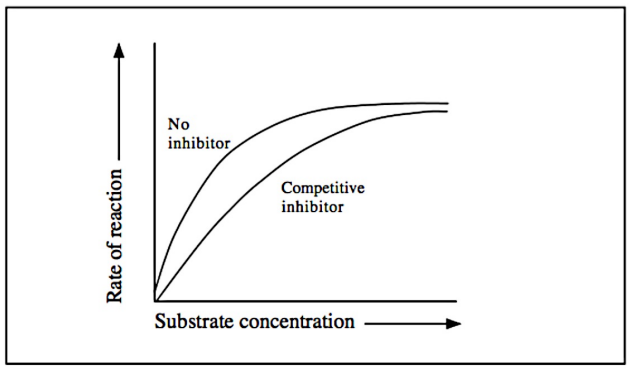



**Typical Exam Questions**

1. Describe and explain the effect of pH, temperature, enzyme concentration etc. on rate of reaction
2. Explain the induced fit hypothesis
3. Explain the role of cofactors

However, if the concentration of the normal substrate is increased, reversible inhibitors are displaced from the active site and the normal enzyme substrate complex can form (Fig 7).

**Fig 7. Effect of increased substrate concentration on reversible competitive inhibition**



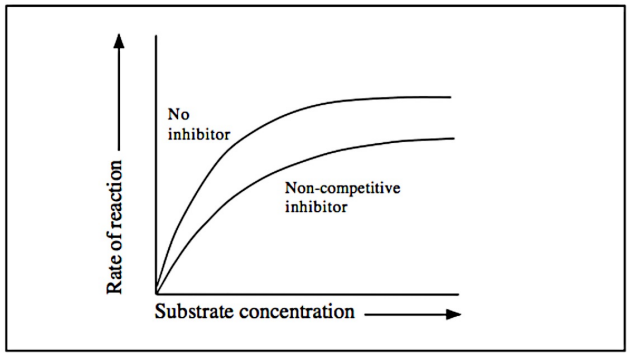

Example 1: arabinose competes with glucose for the active sites on glucose oxidase.

Example 2: oxaloacetate, malonate and pyrophosphate all compete with succinate for the active site of the enzyme succinate dehydrogenase.

Example 3: an individual who swallows methanol is in danger of becoming blind. This is because the methanol – which itself is not toxic – will be metabolised to formaldehyde which is extremely toxic and will cause blindness. At hospital, the individual will be treated with ethanol. The ethanol is structurally similar to methanol and will compete with methanol for the enzyme's active sites. Thus, the metabolism of methanol is slowed down.

**Non-competitive reversible inhibitors** react with the enzyme but not at the active site. They change the shape of the whole enzyme, including the shape of the active site, hence the reaction cannot proceed and no products are formed on those enzymes (Fig 8).

**Fig 8. Effect of increased substrate concentration on non-competitive inhibition**




### (b) Irreversible inhibitors

Irreversible inhibitors bind covalently and permanently to the enzyme, preventing normal enzyme function. For example, Aspirin is an irreversible inhibitor of cycloxygenase, an enzyme involved in the synthesis of prostaglandins. Substances such as mercury, iron and arsenic bind irreversibly to the SH (sulphydryl) group on enzymes.

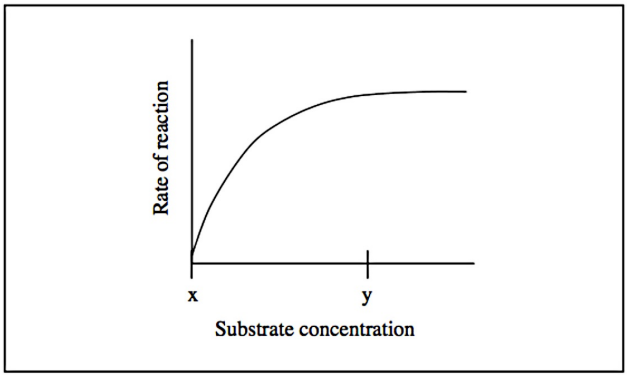
### Practice Questions

1. Define the following terms:

   (a) induced fit hypothesis (3 marks)

   (b) denaturation (3 marks)

2. The graph show the effect of increasing substrate concentration on the rate of an enzyme controlled reaction.




   (a) Explain the shape of the curve between points x and y (2 marks)

   (b) Describe and explain the effect which a competitive reversible inhibitor would have on the rate of this reaction (2 marks)

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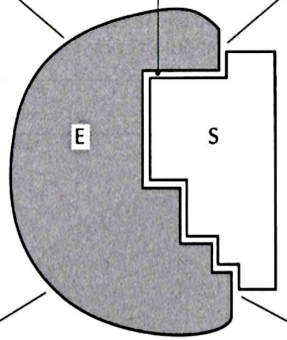
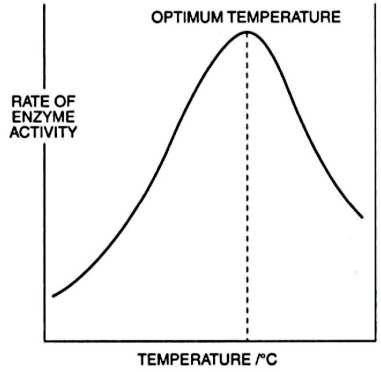
# Factors affecting enzyme activity exert their effects by altering the ease with which an enzyme-substrate complex is formed.

**Any factor which alters the conformation (dependent on tertiary structure) of the enzyme will alter the shape of the active site, affect the frequency of enzyme-substrate complex formation and thus influence the rate of the enzyme-catalysed reaction.**



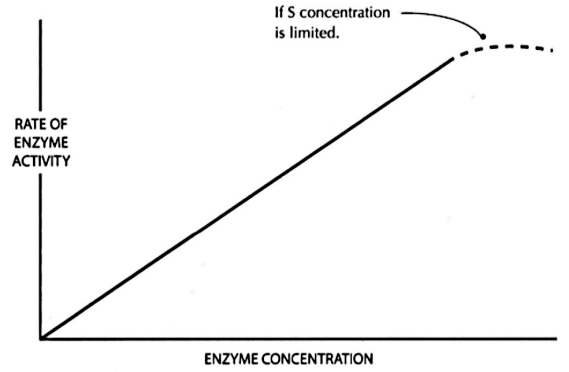

## Effect of temperature



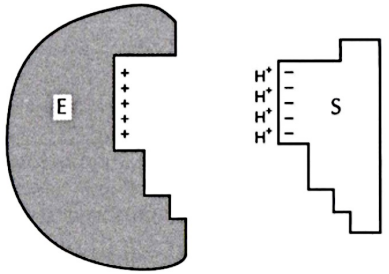

Enzymes have an **optimum temperature** which represents a compromise between **activation** due to increased rate of collision between E and S and **loss of activity** due to denaturation of E molecules and consequent distortion of the active site.

## Effect of enzyme concentration

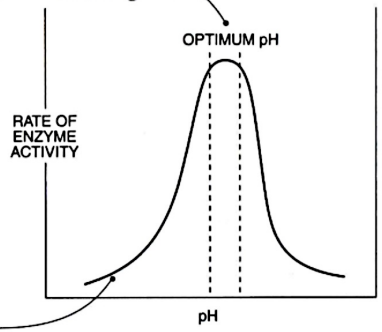
Enzymes are not used up during catalysis, and enzyme molecules can be used over and over again; enzymes therefore work very well at low concentrations. Increasing the concentration of enzyme provides more active sites so the rate of enzyme activity increases **so as an excess of substrate molecules is available.**




## Effect of pH

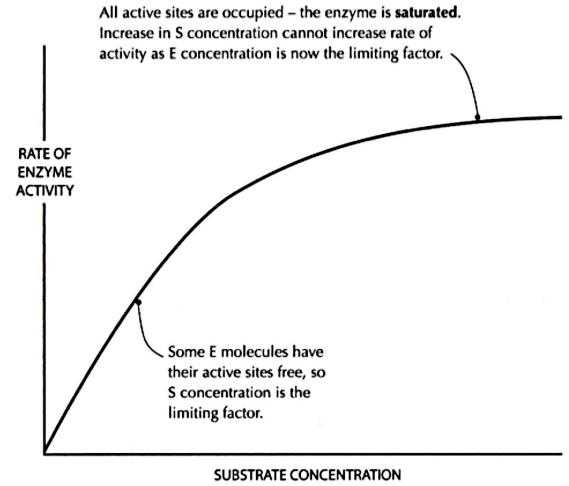



An enzyme has an **optimum pH** which results from the effects of hydrogen ion concentration on the 3-dimensional shape of the enzyme in the active site region.




Greater changes in pH can distort the whole enzyme - DENATURATION - and cause huge loss of enzyme activity.

## Effect of substrate concentration



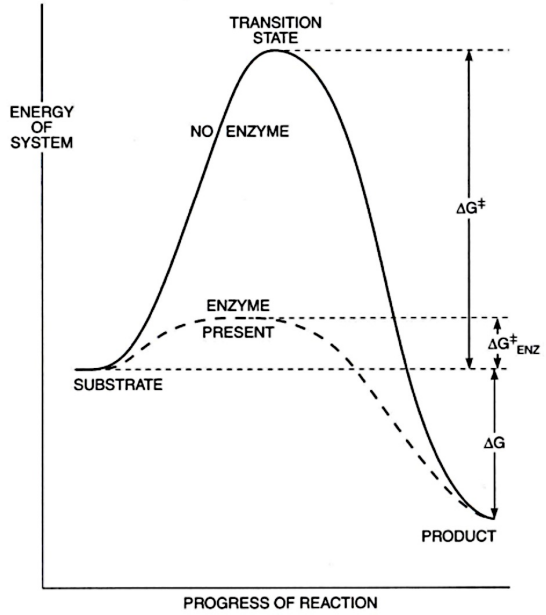
# Catalysis by enzymes

An important step in enzyme catalysis is substrate binding to the active sites.

Enzymes form **enzyme-substrate complexes** which reduce the activation energy for reactions which they catalyse.

Consider the reaction: SUBSTRATE (S) ⟶⟵ PRODUCT (P)

which can be illustrated by a **reaction profile.**



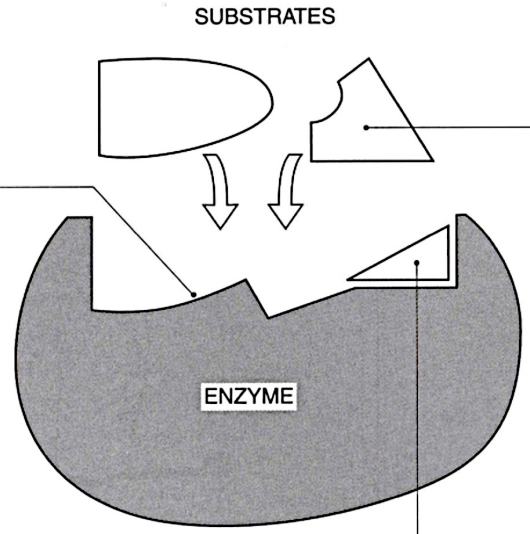

**Effect of enzyme on activation energy**

- Rate of forward reaction, S→P, depends on activation energy and temperature.
- Enzymes act as catalysts by lowering the activation energy ($\Delta G^{\ddagger}$). They do this by providing alternative reaction pathways.
- Enzymes **do not** reduce the overall free energy change ($\Delta G$) for the reaction.
- Large temperature changes cannot be used by cells to change rates of reaction because of possible denaturation.

Summary for overall reaction:

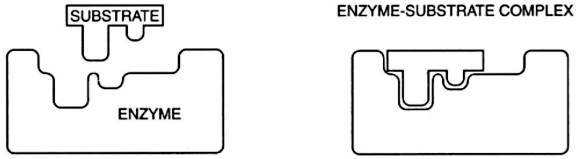
$$E + S \rightleftharpoons [E\text{–}S] \rightleftharpoons E + P$$

Enzyme-substrate complex

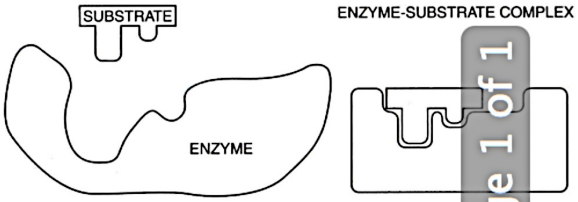



**Stereospecificity: relationship of substrate(s) to active site**

Emil Fischer's **lock and key hypothesis** suggested that the active site and the substrate were **exactly complementary,** i.e. the substrate fits exactly into the active site.




More recent work allowed **Koshland** to propose the **induced fit hypothesis** which suggests that active site and substrate are only fully complementary **after the substrate is bound.**




This latter process of **dynamic recognition** is now the more widely accepted hypothesis. It is supported by modern techniques of imaging which show the enzyme changing shape as the substrate binds it.

Page 1 of 1

**Cofactors are essential for enzyme activity**

Some, such as $Zn^{2+}$ or $Mg^{2+}$, or porphyrin groups such as the **haem** in catalase, may form part of the active site and cannot easily be separated from the enzyme protein: these are commonly called **prosthetic groups.**

Some, such as NAD (nicotinamide adenine dinucleotide), bind temporarily to the active site and actually take part in the reaction.

$$\text{e.g. lactate} + \text{NAD} \xrightleftharpoons{\text{LACTATE DEHYDROGENASE}} \text{pyruvate} + NADH_2$$

Such **coenzymes** shuttle between one enzyme system and another - most are formed from dietary components called **vitamins** (e.g. NAD is formed from niacin, one of the B vitamin complex).

Enzymes are released once the reaction is complete, and are ready for use again. Eventually the enzyme-proteins do lose their shape and must be replaced by the cells.

Some enzymes (e.g. those involved in protein synthesis) are INTRACELLULAR (they are used **inside** the cell). Others (e.g. digestive enzymes) are EXTRACELLULAR (they are secreted and used **outside** the cell.

## Questions on Enzymes

1. Enzymes are described as being globular proteins, what does this mean?

2. What bonds maintain the structure of a globular protein?

3. Enzymes are also described as biological catalysts. Why is the term biological used? What are the features of catalysts?

4. Draw an enzyme and label the active site. Annotate the diagram to explain the significance of the active site.

5. Draw an enzyme substrate complex and explain its significance.

6. Distinguish using diagrams the features of the two main proposed models for enzyme action.

   I The Lock and key
   II The Induced Fit

7. Define the following terms:
   - Activation energy
   - Exergonic reaction
   - Endergonic reaction
   - Catabolic reaction
   - Anabolic reaction

8. Sketch two energy profiles, one for an endergonic reaction and one for an exergonic reaction. Are anabolic or catabolic reactions exergonic?

9. Sketch a graph to show how substrate concentration affects the rate of an enzyme controlled reaction. Annotate the graph to explain what is happening at each stage; ensure that you use the term maximum turnover number.

10. Sketch a graph to show how temperature affects the rate of an enzyme controlled reaction. Annotate your graph to explain what is occurring at each stage. Make sure that you include the terms kinetic energy, successful collisions, bonds, optimum temperature, active site and denaturation.

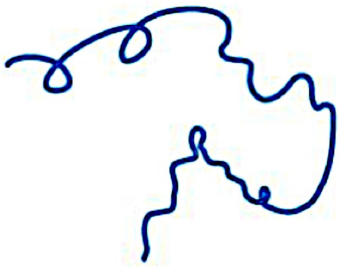
11. What is the $Q_{10}$ coefficient for enzyme reactions?

12. Sketch a graph to show how pH affects the rate of an enzyme controlled reaction. Annotate your graph to explain what is occurring at each stage.

13. Using the information on the beginning of the next page – explain what happens when an enzyme denatures. Explain how temperatures above the optimum or extreme changes in pH can bring about denaturation.

The tertiary structure of the enzyme molecule is held by many weak bonds

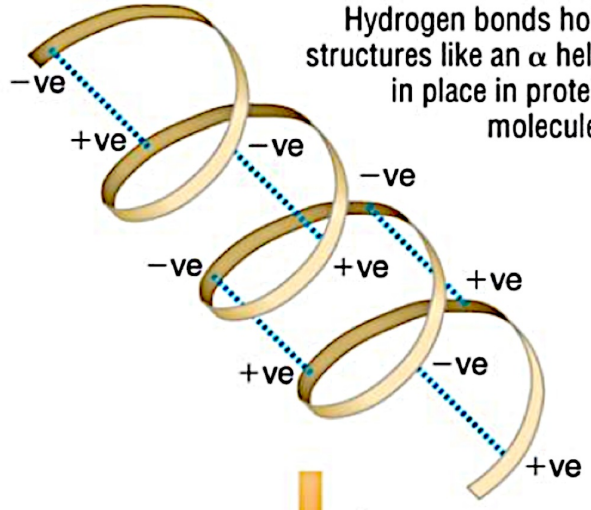
Heat energy



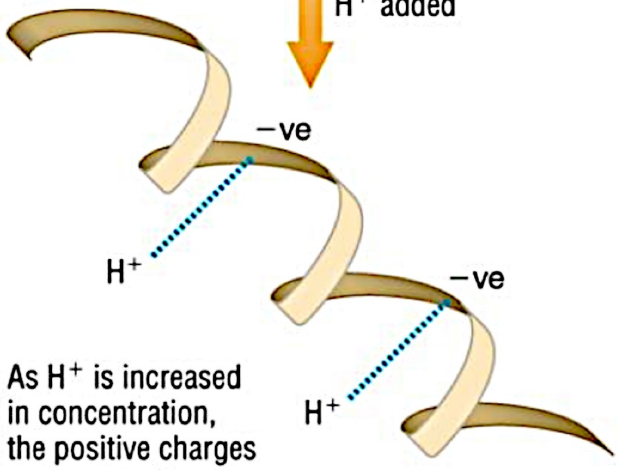
If enough of these weak bonds are broken by heating, the tertiary structure 'unravels'. The active site shape is lost and the enzyme is denatured

**Note**: this does not affect the primary structure i.e. covalent peptide bonds are not broken

Hydrogen bonds hold structures like an α helix in place in protein molecules




$H^+$ added




As $H^+$ is increased in concentration, the positive charges are attracted to the negative charges on the α helix and so 'replace' the hydrogen bonds

**14.** Sketch a graph to show what happens when the enzyme concentration is increased in an enzyme controlled reaction. On the graph draw 2 lines, one that shows what happens when substrate is fixed and one, which shows what happens when substrate concentration is in excess.

**15.** Metabolism is described as being a series of enzyme-controlled reactions. Give 3 examples of metabolic pathways found in humans.

**16.** What are meant by the terms intracellular and extracellular? Give 3 examples of enzymes that act on an intracellular level and 3 examples of enzyme that will act extracellular level.